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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/695,546	BARNES ET AL.	
	Examiner	Art Unit	
	Stephen Kapushoc	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 January 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *2-10,12-21,24 and 25* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) *2-10, 12-21, 24 and 25* is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims 2-10, 12-21, 24 and 25 are pending and examined on the merits.

Please Note: The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

This Office Action is in reply to Applicants' correspondence of 1/16/2007.

Applicants' remarks and amendments have been fully and carefully considered but are not found to be sufficient to put the application in condition for allowance. Any new grounds of rejection presented in this Office Action are necessitated by Applicants' amendments. Any rejections or objections not reiterated herein have been withdrawn in light of the amendments to the claims or as discussed in this Office Action.

This Action is made **FINAL**.

Withdrawn Objection to the Specification

1. It is noted that the amendment to the specification of 1/16/2007 does not appear to address the objection as set forth in the Office Action of 6/20/2006, however upon further consideration of the phrase 'any or the purification methods' the objection is **WITHDRAWN**, where the phrase indicates that any method may be used, or the specifically described purification methods may be used.

Withdrawn Claim Objections

2. The objection to claims 1, 21, and 24, as set forth in the previous Office Action, is **WITHDRAWN** in light of the cancellation of claim 1 and the amendments to claims 21 and 24.

Withdrawn Claim Rejections - 35 USC § 112 2nd –Indefiniteness

3. The rejections of claims 4-6 and 14-19 under 35 U.S.C. 112, second paragraph, as being indefinite are **WITHDRAWN** in light of the amendments to the claims.

New Claim Rejections - 35 USC § 112 – New Matter

4. Claim 25 rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection.

Claims 25 requires selectively amplifying the AHAS3 gene using an 'AHAS3 reverse primer having a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO: 10' (part b of claim 25). While the breadth of the sequence of the required primer is noted, where the indefinite article 'a' in the phrase limits the required primer only to having any (i.e. as few as two contiguous nucleotides for 'a sequence') nucleotides from the recited SEQ ID NO: 10, the specification as originally filed does not provide for using SEQ ID NO: 10 or portions thereof in the selective amplification of the AHAS3 gene. The specification refers to SEQ ID NO: 10 as a reverse primer for amplification of the AHAS1 gene (i.e. p.6 ¶[023]; p.9 ¶[033]; p.16 ¶[054];) and specifically SEQ ID NO: 14 as a reverse primer for amplification of the AHAS3 gene (i.e. p.6 ¶[023]; p.11 ¶[039]; p.18 ¶[058]). As such, the specification as originally filed does not appear to disclose or contemplate the use of SEQ ID NO: 10 or portions thereof as a reverse primer for selectively amplifying the AHAS3 gene.

Maintained Claim Rejections - 35 USC § 103
With new grounds as necessitated by amendment

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 2-9, and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rutledge et al (1991, as cited in the IDS) in view of Sathasivan et al (1991), and Shi et al (1996).

Rutledge et al teaches the nucleic acid and deduced amino acid sequence of the *Brassica AHAS1* gene (Fig. 2A). The reference teaches that DNA was isolated from leaf nuclei, relevant to claims 2 and 3 step (a), and claim 24 step (a). The reference also teaches that imidazolinone herbicides act through inhibition of AHAS (p.39, left column, last paragraph), and further teaches that herbicide resistance in *Brassica* mutants results from two unlinked alleles, and that the effect of combining the alleles in a hybrid line is additive for imidazolinone resistance. The reference teaches that *AHAS1* is an imidazolinone resistance allele (p.39, left column, last paragraph), and concludes that the sequences of the *AHAS* genes provides the basic information essential for the analysis of *Brassica* mutants with resistance to herbicides that act on *AHAS* (p.39, right column, last paragraph). Regarding the primers recited in claims 2, 3, and 24, positions 2442-2463 of the sequence of the AHAS1 gene taught by Rutledge et

al (Fig 2A) includes the 21 of the 22 nucleotides of SEQ ID NO: 9 (relevant to claims 2 and 24), and positions 4655-4676 of the sequence of the AHAS1 gene taught by Rutledge et al (Fig 2A) are complementary to the 21 of the 22 nucleotides of SEQ ID NO: 10 (relevant to claim 3).

Regarding claim 4, the sequence of the AHAS1 gene taught by Rutledge et al (Fig 2A) includes the sequence of the primer set forth in SEQ ID NO: 11 of the instant specification. Nucleotides 4364-4384 of the AHAS3 gene taught by Rutledge et al are identical to nucleotides 1-21 of SEQ ID NO: 11.

Regarding claim 5, the sequence of the AHAS1 gene taught by Rutledge et al (Fig 2A) includes the sequence of the primer set forth in SEQ ID NO: 12 of the instant specification. Nucleotides 4589-4609 of the AHAS3 gene taught by Rutledge et al are complementary to nucleotides 1-21 of SEQ ID NO: 12.

Rutledge et al does not teach the nature of the mutation in the AHAS1 gene (PM1) that confers resistance to imidazolinone.

Sathasivan et al teaches the analysis of an *A. thaliana* mutation in the acetolactate synthase gene (referred to within the reference as ALS, which is an art recognized synonym for AHAS). The reference teaches that the mutation provides the molecular basis for imidazolinone resistance in *A. thaliana* (p.1044 – Abstract). Sathasivan et al teaches the specific nature of the *A. thaliana* mutation responsible for herbicide resistance as a G to A single-point mutation at nucleotide 1958 of the coding sequence, which predicts a serine to asparagine substitution at amino acid 653 (p.1044, left column, last paragraph; Fig. 2; Table 1). The reference also provides an alignment

indicating the conservation of the deduced amino acid residues in the acetolactate synthase gene near the mutation site conferring imidazolinone resistance (Fig. 3). Based on the teachings of Sathasivan et al (i.e. the sequence provided in Fig 2 and the alignment provided in Fig. 3), and the teaching of Rutledge et al (i.e.: the sequence of the AHAS1 gene together with the teaching that a mutation in AHAS1 provides imidazolinone resistance), it is evident that the *A. thaliana* G to A mutation taught by Sathasivan is equivalent to the PM1 mutation claimed in the instant application. The reference also teaches that similar mutations at corresponding nucleotide positions of other acetolactate synthase genes can confer imidazolinone resistance (p.1049, left column, last paragraph).

Sathasivan et al does not provide a method for the detection of the PM1 mutation comprising analysis of intramolecular interactions of an amplification product created by nested PCR.

Shi et al teaches a method for the analysis of mutations using single-strand conformational polymorphism (SSCP) analysis. Relevant to steps (b) and (d) of claims 2 and 3 and steps (b) and (d) of claim 24, the reference teaches that PCR products of target regions that were amplified using a first set of primers were subsequently reamplified using a second set of primers (p.272 – Nested PCR with exonuclease I selection; Table 1). The method of amplification taught by Shi et al comprises a first selective amplification of a gene region of interest using forward and reverse primers (relevant to step (b) of claims 2, 3 and 24), followed by a step of removing remaining outer primers using exonuclease I (relevant to step (c) of claims 2, 3, and 24), and a

subsequent amplification of a portion of the gene having a mutation using forward and reverse primers nested within the primers used for the first amplification (relevant to step (d) of claims 2, 3, and 24). The reference further teaches analysis of the amplified products using SSCP by denaturing the amplification product to produce single stranded polynucleotides that are allowed to adopt particular conformations and the detection of mutations based on mobility of the strands through a substrate (p.273 – SSCP analysis; p.271, right col., last paragraph; Fig. 1), relevant to steps (e) and (f) of claims 2, 3, and 24.

Regarding claims 6 and 7, the reference teaches the incorporation of a radioactive label into the amplification product (p.273, left col., first full paragraph).

Regarding claim 8, the reference teaches the analysis of amplification products using a substrate that is polyacrylamide (p.273 – SSCP analysis).

Regarding claim 9, the reference teaches detection using electrophoresis (p.273 – SSCP analysis).

Thus it would have been prima facie obvious to one of skill in the art at the time the invention was made to have combined the information and methods provided in the cited references to have created the claimed invention of a method to assay imidazolinone resistance in a *Brassica* plant conferred by the PM1 mutation of the *AHAS1* gene. One would have been motivated to develop such an assay to efficiently determine the presence of herbicide resistance in a plant using molecular techniques. One would have had a reasonable expectation of success because the cited references teach both the general aspects of the properties responsible for imidazolinone

resistance, as well as the specific molecular characteristics that confer imidazolinone herbicide resistance. Rutledge et al teaches that an allele of the *AHAS1* gene is responsible for imidazolinone resistance in a *B. napus* (p.39, right column, last paragraph). Rutledge et al further teaches the nucleic acid sequence and deduced amino acid sequence of the *B. napus AHAS1* gene (Fig. 2A). Sathasivan et al teaches the identification of a G to A (serine to asparagine) mutation in the *A. thaliana* ALS gene and provides an amino acid alignment indicating that this mutation is equivalent to the PM1 mutation of the instant application, and teaches that the mutation is applicable to *Brassica*. One would have been motivated to use the particular methods of Shi et al based on the assertion of Shi et al that the method is simple, effective, rapid, and inexpensive (p.276, right col., lns.22-24) and provides improved final results in nucleic acid amplification based methods (p.274, left col., lns.12-13).

Regarding the specific primers recited in claim 2 (a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO: 9), claims 3 (a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO: 10), and claims 4 and 5 (SEQ ID NO: 11 and 12), it would be obvious to use primers comprising or consisting of these sequences because these sequences are taught by Rutledge et al, and Sathasivan et al teaches the position of the PM1 mutation as nested between these nucleic acid sequences. It is particularly noted that in the recitation of required primers in claims 2, 3, and 24, the claims broadly recite, for example, an AHAS1 forward primer having a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:9, where the indefinite article 'a' refers to any portion of the recited positions of the recited SEQ ID NO. Further, the method of Shi et al teaches using

amplification primers that flank a region of interest for SSCP analysis. It would thus be obvious to use any primers (based on the sequences provided by Rutledge et al) flanking the position of the PM1 mutation (as taught by Sathasivan et al), including primers having a sequence as set forth in SEQ ID NO: 9, 10, 11, or 12 for analysis of the PM1 mutation using SSCP (as taught by Shi et al).

Regarding claim 24, Rutledge et al also teaches combining different *AHAS* mutants in a hybrid line. It would thus be obvious to select of plants identified as having the PM1 mutation for further breeding (relevant to step (g) of claim 24), to create plants with higher herbicide resistance (p.39, left col., last paragraph).

Response to Remarks

Applicants have indicated (page 12 of Remarks) that the rejection of claims under 35 USC 103 as obvious in view of the teachings of Rutledge et al in view of Sathasivan et al, and Shi et al is obviated by the amendments to the claims. It is noted that the subject matter of claims 2 and 3 as originally presented was indicated as allowable in the Office Action of 6/20/2006. However, the previously presented claims required, for example in previously presented claim 2, 'wherein the AHAS1 forward primer has the sequence set forth in SEQ ID NO: 9' thus requiring a primer comprising the sequence of SEQ ID NO: 9. As amended, claim 2 requires a primer 'having a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO: 9', where, as set forth in the rejection above, the indefinite article 'a' refers to any portion of the recited positions of the recited SEQ ID NO, thus the claims require only any primer having at least 2 contiguous nucleotides, thus 'a sequence', as presented in SEQ ID NO: 9.

The rejection, as set forth above, is **MAINTAINED.**

7. Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Rutledge et al (1991, as cited in the IDS) in view of Sathasivan et al (1991) and Shi et al (1996) as applied to claims 1, 4-9, and 24, and further in view of Hattori et al (1995, as cited in the IDS).

Rutledge et al in view of Sathasivan et al and Shi et al teaches a method for assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM1 mutation of the *AHAS1* gene comprising all of the limitations of claim 1, from which the rejected claim depends.

In addition to the sequence of the *AHAS1* gene, Rutledge et al teaches the nucleic acid and deduced amino acid sequence of the *Brassica AHAS3* gene (Fig. 2C). The reference also teaches that imidazolinone herbicides act through inhibition of *AHAS* (p.39, left column, last paragraph), and further teaches that herbicide resistance in *Brassica* mutants results from two unlinked alleles, and that the effect of combining the alleles in a hybrid line is additive for imidazolinone resistance. The reference teaches that in addition to *AHAS1*, *AHAS3* is an imidazolinone resistance allele (p.39, left column, last paragraph).

Rutledge et al does not teach the nature of the mutation in the AHAS3 gene (PM2) that confers resistance to imidazolinone.

Hattori et al teaches the analysis of the AHAS3 gene from imidazolinone-resistant mutant *Brassica* cells. The reference teaches that the AHAS3 gene from the

mutant cells was cloned and sequenced, and the sequence of the gene from the mutant was compared to the wild-type AHAS3 sequence (p.420, right column, l.28). Hattori teaches the identification of a single basepair change (G to T) in AHAS3 that predicts a tryptophan to leucine amino acid change (p.421, left column, last paragraph), and provides a comparative alignment of deduced amino acid sequences in the region of the AHAS3 mutation responsible for herbicide resistance (p.421 Fig. 2). Based on the alignment provided in Fig. 2 of Hattori et al, and the sequence of the AHAS3 gene provided by Rutledge et al, it is evident that the G to T mutation taught by Hattori is equivalent to the PM2 mutation claimed in the instant application. Hattori concludes that the identified mutation site in the AHAS3 gene is involved in the binding of imidazolinone herbicides, and teaches that the recovery of the same mutation in tobacco and *B. napus*. Further relevant to step (b) of claim 1 and step (b) of claim 25, Hattori et al teaches amplification of the *AHAS3* gene using a forward and reverse primer (p.420 – Isolation of *AHAS1* and *AHAS3* genes from herbicide resistant C20 line of *B. napus* callus).

It would have been prima facie obvious at the time the invention was made to have combined the methods of Rutledge et al in view of Sathasivan et al and Shi et al to include further detection of the PM2 mutation taught by Hattori et al. One would have been motivated to detect both mutations based on the teachings of Rutledge et al that combination of the two alleles in a single hybrid plant has an additive effect on imidazolinone resistance (p.39, left col., last paragraph), and the teachings of Hattori et al that the mutation taught by Hattori et al confers resistance to imidazolinone.

Response to Remarks

Applicants have indicated (page 12 of Remarks) that the rejection of claims under 35 USC 103 as obvious in view of the teachings of Rutledge et al in view of Sathasivan et al, and Shi et al, and further in view of Hattori et al is obviated by the amendments to the claims. As discussed above, the amended subject matter of claims 2 and 3, from which rejected claim 10 depends, broadly require a primer 'having a sequence as set forth in nucleotides 1 to 22 of' SEQ ID NO: 9 or 10 (claim 2 or 3, respectively), where, as set forth in the rejection above, the indefinite article 'a' refers to any portion of the recited positions of the recited SEQ ID NO. Thus independent claims 2 and 3 require only any primer having at least 2 contiguous nucleotides, thus 'a sequence', as presented in the recited SEQ ID NO: 9 or 10.

The rejection, as set forth above, is **MAINTAINED.**

8. Claims 12-20 and 25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Rutledge et al (1991, as cited in the IDS) in view of Hattori et al (1995, as cited in the IDS), Liu et al (2001, US Patent 6,207,425), and Shi et al (1996).

Rutledge et al teaches the nucleic acid and deduced amino acid sequence of the *Brassica AHAS3* gene (Fig. 2C). The reference teaches that DNA was isolated from leaf nuclei, relevant to claims 12 and 13 step (a), and claim 25 step (a). The reference also teaches that imidazolinone herbicides act through inhibition of *AHAS* (p.39, left column, last paragraph), and further teaches that herbicide resistance in *Brassica* mutants results from two unlinked alleles, and that the effect of combining the alleles in

a hybrid line is additive for imidazolinone resistance. The reference teaches that *AHAS3* is an imidazolinone resistance allele (p.39, left column, last paragraph), and concludes that the sequences of the *AHAS* genes provides the basic information essential for the analysis of *Brassica* mutants with resistance to herbicides that act on *AHAS* (p.39, right column, last paragraph). Regarding the primers recited in claims 12, 13, and 25, positions 173-194 of the sequence of the AHAS3 gene taught by Rutledge et al (Fig 2C) includes the 21 of the 22 nucleotides of SEQ ID NO: 13 (relevant to claims 13), and also comprises a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO: 10 (relevant to claim 25; i.e.: positions 175-177 of AHAS3 are 5'-CAA-3', where that is a sequence of positions 11-13 of SEQ ID NO: 10), and positions 2396-2418 of the sequence of the AHAS3 gene taught by Rutledge et al (Fig 2C) are complementary to the 22 of the 23 nucleotides of SEQ ID NO: 14 (relevant to claim 13).

Regarding claim 14, the sequence of the AHAS3 gene taught by Rutledge et al (Fig 2C) includes the sequence of the primer set forth in SEQ ID NO: 15 of the instant specification. Nucleotides 1730-1748 of the AHAS3 gene taught by Rutledge et al are identical to nucleotides 1-19 of SEQ ID NO: 15.

Regarding claim 15, the sequence of the AHAS3 gene taught by Rutledge et al (Fig 2C) includes the sequence of the primer set forth in SEQ ID NO: 16 of the instant specification. Nucleotides 2117-2099 of the AHAS3 gene taught by Rutledge et al are complementary to nucleotides 1-19 of SEQ ID NO: 16.

Regarding claim 16, the sequence of the AHAS3 gene taught by Rutledge et al (Fig 2C) includes the sequence of the primer set forth in SEQ ID NO: 17 of the instant

specification. Nucleotides 1907-1924 of the AHAS3 gene taught by Rutledge et al are identical to nucleotides 1-18 of SEQ ID NO: 17.

Regarding claim 16, the sequence of the AHAS3 gene taught by Rutledge et al (Fig 2C) includes the portion of the sequence of the primer set forth in SEQ ID NO: 18 of the instant specification that is not specific for the PM2 mutation. Nucleotides 1905-1923 of the AHAS3 gene taught by Rutledge et al are identical to nucleotides 1-19 of SEQ ID NO: 18.

Rutledge et al does not teach the nature of the mutation in the AHAS3 gene (PM2) that confers resistance to imidazolinone.

Hattori et al teaches the analysis of the AHAS3 gene from imidazolinone-resistant mutant *Brassica* cells. The reference teaches that the AHAS3 gene from the mutant cells was cloned and sequenced, and the sequence of the gene from the mutant was compared to the wild-type AHAS3 sequence (p.420, right column, l.28). Hattori teaches the identification of a single basepair change (G to T) in AHAS3 that predicts a tryptophan to leucine amino acid change (p.421, left column, last paragraph), and provides a comparative alignment of deduced amino acid sequences in the region of the AHAS3 mutation responsible for herbicide resistance (p.421 Fig. 2). Based on the alignment provided in Fig. 2 of Hattori et al, and the sequence of the AHAS3 gene provided by Rutledge et al, it is evident that the G to T mutation taught by Hattori is equivalent to the PM2 mutation claimed in the instant application. Hattori concludes that the identified mutation site in the AHAS3 gene is involved in the binding of imidazolinone herbicides, and teaches that the recovery of the same mutation in

tobacco and *B. napus*. Further relevant to step (b) of claims 12 and 13 and step (b) of claim 25, Hattori et al teaches amplification of the *AHAS* genes using a forward and reverse primers (p.420 – Isolation of *AHAS1* and *AHAS3* genes from herbicide resistant C20 line of *B. napus* callus).

Hattori does not teach performing second and third amplification steps using region specific forward and reverse primers and primers specific for the wild type or PM2 mutation allele of the *AHAS3* gene.

Liu et al teaches a method for the bidirectional PCR amplification of specific alleles (Bi-PASA) for the detection of mutations (Figure 1A; col.4 lns. 30-51), relevant to steps (d) and (e) of claims 12, 13, and 25. The reference teaches that amplification is carried out using region specific forward and reverse primers (identified as 'P' and 'Q' in the reference) which provide a positive control (col. 4 lns.53-54), as well as allele specific primers (identified as 'A' and 'B' in the reference) which are selective for the mutant and wildtype alleles of the analyzed gene (col.4 lns.54-57). The reference further teaches that the method can be performed using all four primers in one reaction, or as separate reactions using different combinations of useful primers (col.5 lns 31-36), and specifically teaches the analysis of reactions containing primers PQB or PQA (e.g.: Fig. 3A lanes 16 and 17, respectively). Relevant to claims 18 and 19, the reference teaches incorporation of a radioactive label into the amplified nucleic acid for purposes of analysis of the amplification products (col.3 lns.24-30; Fig 2; col.6 ln.15). Relevant to claim 20, the reference teaches analysis of amplification products by electrophoresis (col.6 lns.15-17).

Liu et al does not teach a method comprising the removal of primers from a previous gene amplification step, prior to a subsequent PCR amplification.

Shi et al teaches a method for the analysis of mutations nested PCR and removal of unincorporated primers. Relevant to steps (b) and (d) of claim 12 and 13 and steps (b) and (d) of claim 25, the reference teaches that PCR products of target regions that were amplified using a first set of primers were subsequently reamplified using a second set of primers (p.272 – Nested PCR with exonuclease I selection; Table 1). The method of amplification taught by Shi et al comprises a first selective amplification of a gene region of interest using forward and reverse primers (relevant to step (b) of claims 12, 13, and 25), followed by a step of removing remaining outer primers using exonuclease I (relevant to step (c) of claims 12, 13, and 25), and a subsequent amplification of a portion of the gene having a mutation using forward and reverse primers nested within the primers used for the first amplification (relevant to step (d) of claims 12, 13 and 25).

Thus it would have been prima facie obvious to one of skill in the art at the time the invention was made to have combined the information and methods provided in the cited references to have created the claimed invention of a method to assay imidazolinone resistance in a *Brassica* plant conferred by the PM2 mutation of the *AHAS3* gene. One would have been motivated to develop such an assay to efficiently determine the presence of herbicide resistance in a plant using molecular techniques. One would have had a reasonable expectation of success because the cited references teach both the general aspects of the properties responsible for imidazolinone

resistance, as well as the specific molecular characteristics that confer imidazolinone herbicide resistance. Rutledge et al teaches that an allele of the *AHAS3* gene is responsible for imidazolinone resistance in a *B. napus* (p.39, right column, last paragraph). Rutledge et al further teaches the nucleic acid sequence and deduced amino acid sequence of the *B. napus* AHAS3 gene (Fig. 2C). Hattori et al teaches the identification of a G to T (tryptophan to leucine) mutation in the *B. napus* AHAS3 gene responsible for imidazolinone resistance that is equivalent to the PM2 mutation of the instant application. One would have been motivated to use the particular techniques of Liu et al and Shi et al based on the teachings of Liu et al that the method is rapid and particularly useful for analysis of single-base change mutants (col. 2 lns.49-61) and the teachings of Shi et al that nested PCR methods using removal of primers prior to the second amplification are simple, effective, rapid, and inexpensive (p.276, right col., lns.22-24) and provide improved final results in nucleic acid amplification based methods (p.274, left col., lns.12-13). Combining the methods of Liu et al and Shi et al would create a method in which the gene of interest is first amplified using a first primer pair comprised of gene specific forward and reverse primers, followed by removal of primers, and then diagnostic amplifications using forward and reverse primers that are nested within the first primer pair and wild type or mutation specific primers.

Regarding the specific primers recited in claims 14, 15, and 17 (SEQ ID NO: 15, 16, and 18), it would be obvious to use primers comprising or consisting of these sequences because these sequences are taught by Rutledge et al; and Hattori et al teaches the position of the PM2 mutation as nested between the nucleic acid

sequences of SEQ ID NO: 15 and 16, and the presence of the mutation creates a sequence comprising SEQ ID NO: 18. Regarding the specific primers recited in claim 12 (having a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO: 13), claim 13 (having a sequence as set forth in nucleotides 1 to 23 of SEQ ID NO: 14), and claim 25 having a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO: 10), it would be obvious to use primers comprising the broadly required sequence elements based on the teachings of the AHAS3 sequence of Rutledge et al. It is particularly noted that in the recitation of required primers in claims 12, 13, and 25, the claims broadly recite, for example, an AHAS3 forward primer having a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO: 13, where the indefinite article 'a' refers to any portion of the recited positions of the recited SEQ ID NO. It would thus be obvious to use any primers (based on the sequences provided by Rutledge et al) flanking the position of the PM2 mutation (as taught by Hattori et al), including primers having a sequence as set forth in SEQ ID NO: 13, 14, or 10 for analysis of the PM2 mutation.

Further, the method of Liu et al teaches using amplification primers that flank a region of interest and an allele specific primer with a discriminating nucleotide at its 3'-end for mutation analysis. It would thus be obvious to use any primers (based on the sequences provided by Rutledge et al) flanking the position of the PM2 mutation (as taught by Hattori et al), including primers comprising or consisting of SEQ ID NO: 14 and 15, as well as any primer with a PM2 specific nucleotide at its 3'-end, including a primer comprising or consisting of SEQ ID NO: 18, for analysis of the PM2 mutation using allele specific amplification.

Regarding claim 25, Rutledge et al also teaches combining different *AHAS* mutants in a hybrid line. It would thus be obvious to select of plants identified as having the PM2 mutation for further breeding (relevant to step (g) of claim 25), to create plants with higher herbicide resistance (p.39, left col., last paragraph).

Response to Remarks

Applicants have indicated (page 12 of Remarks) that the rejection of claims under 35 USC 103 as obvious in view of the teachings of Rutledge et al in view of Hattoriet al, Liu et al, and Shi et al is obviated by the amendments to the claims. It is noted that the subject matter of claims 12 and 13 as originally presented was indicated as allowable in the Office Action of 6/20/2006. However, the previously presented claims required, for example in previously presented claim 12, 'wherein the AHAS3 forward primer has the sequence set forth in SEQ ID NO: 13' thus requiring a primer comprising the sequence of SEQ ID NO: 13. As amended, claim 12 requires a primer 'having a sequence as set forth in nucleotides 1 to 22 of SEQ ID NO: 13, where, as set forth in the rejection above, the indefinite article 'a' refers to any portion of the recited positions of the recited SEQ ID NO, thus the claims require only any primer having at least 2 contiguous nucleotides, thus 'a sequence', as presented in SEQ ID NO: 13.

The rejection, as set forth above, is **MAINTAINED.**

9. Claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over Rutledge et al (1991, as cited in the IDS) in view of Hattori et al (1995, as cited in the

IDS), Liu et al (2001, US Patent 6,207,425), and Shi et al (1996), as applied to claims 11, 14-20 and 25, and further in view of Sathasivan et al (1991).

Rutledge et al in view of Hattori et al, Liu et al, and Shi et al teaches a method for assaying a *Brassica* plant for imidazolinone herbicide tolerance conferred by the PM2 mutation of the *AHAS3* gene comprising all of the limitations of claim 11, from which the rejected claim depends.

Shi et al additionally teaches analysis of the amplified products using SSCP by denaturing the amplification product to produce single stranded polynucleotides that are allowed to adopt particular conformations and the detection of mutations based on mobility of the strands through a substrate (p.273 – SSCP analysis; p.271, right col., last paragraph; Fig. 1), relevant to steps (j) and (k) of claim 21.

In addition to the sequence of the *AHAS3* gene, Rutledge et al teaches the nucleic acid and deduced amino acid sequence of the *Brassica AHAS1* gene (Fig. 2A). The reference also teaches that imidazolinone herbicides act through inhibition of *AHAS* (p.39, left column, last paragraph), and further teaches that herbicide resistance in *Brassica* mutants results from two unlinked alleles, and that the effect of combining the alleles in a hybrid line is additive for imidazolinone resistance. The reference teaches that in addition to *AHAS3*, *AHAS1* is an imidazolinone resistance allele (p.39, left column, last paragraph).

Rutledge et al does not teach the nature of the mutation in the *AHAS1* gene (PM1) that confers resistance to imidazolinone.

Sathasivan et al teaches the analysis of an *A. thaliana* mutation in the acetolactate synthase gene (referred to within the reference as ALS, which is an art recognized synonym for AHAS). The reference teaches that the mutation provides the molecular basis for imidazolinone resistance in *A. thaliana* (p.1044 – Abstract). Sathasivan et al teaches the specific nature of the *A. thaliana* mutation responsible for herbicide resistance as a G to A single-point mutation at nucleotide 1958 of the coding sequence, which predicts a serine to asparagine substitution at amino acid 653 (p.1044, left column, last paragraph; Fig. 2; Table 1). The reference also provides an alignment indicating the conservation of the deduced amino acid residues in the acetolactate synthase gene near the mutation site conferring imidazolinone resistance (Fig. 3). Based on the teachings of Sathasivan et al (i.e. the sequence provided in Fig 2 and the alignment provided in Fig. 3), and the teaching of Rutledge et al (i.e.: the sequence of the AHAS1 gene together with the teaching that a mutation in AHAS1 provides imidazolinone resistance), it is evident that the *A. thaliana* G to A mutation taught by Sathasivan is equivalent to the PM1 mutation claimed in the instant application. The reference also teaches that similar mutations at corresponding nucleotide positions of other acetolactate synthase genes can confer imidazolinone resistance (p.1049, left column, last paragraph).

It would have been prima facie obvious at the time the invention was made to have combined the methods of Rutledge et al in view of Hattori et al, Liu et al, and Shi et al to have further included detection of the PM1 mutation taught by Sathasivan et al. One would have been motivated to detect both mutations based on the teachings of

Rutledge et al that combination of the two alleles in a single hybrid plant has an additive effect on imidazolinone resistance (p.39, left col., last paragraph), and the assertion of Sathasivan et al that the mutation taught by Sathasivan et al confers imidazolinone resistance. One would have been motivated to use the SSCP method of Shi et al based on the assertion of Shi et al that the method is simple, effective, rapid, and inexpensive (p.276, right col., lns.22-24).

Response to Remarks

Applicants have indicated (page 12 of Remarks) that the rejection of claims under 35 USC 103 as obvious in view of the teachings of Rutledge et al in view of Hattori et al, Liu et al, and Shi et al (1996), and further in view of Sathasivan et al is obviated by the amendments to the claims. As discussed above, the amended subject matter of claims 12 and 13, which rejected claim 21 depends from, broadly requires a primer 'having a sequence as set forth in nucleotides 1 to 22 of' SEQ ID NO: 13 or 14 (claim 12 or 13, respectively), where, as set forth in the rejection above, the indefinite article 'a' refers to any portion of the recited positions of the recited SEQ ID NO. Thus independent claims 12 and 13 require only any primer having at least 2 contiguous nucleotides, thus 'a sequence', as presented in the recited SEQ ID NO: 13 or 14.

The rejection, as set forth above, is **MAINTAINED.**

Requirement For Information Under 37 C.F.R. 1.105

10. The Declaration of Dwight More, provided under 37 CFR 1.132 satisfies the Requirement For Information Under 37 CFR 1.105 as requested in the previous Office Action.

Maintained Double Patenting

11. Claims 2-10, 12-21, 24 and 25 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-5 of copending Application No. 10/695,089 in view of Shi et al (1996) and Liu et al (2001, US Patent 6,207,425.

The claims of the conflicting application are drawn to methods for determining the presence of the PM1 and PM2 mutations in genomic DNA isolated from *B. napus*. The rejected claims of the instant application are drawn to the analysis of the same mutations in the same genes of the same species of plants.

The claims of the copending application do not specifically recite the particular methods for mutation analysis (i.e. nested PCR SSCP, and nested PCR bi-directional PCR amplification of specific alleles). However, such methods were known in the art at the time the invention was made.

The art of Shi et al teaches a method for the analysis of mutations using single-strand conformational polymorphism (SSCP) analysis. The reference teaches that PCR products of target regions that were amplified using a first set of primers were subsequently reamplified using a second set of primers (p.272 – Nested PCR with

exonuclease I selection; Table 1). The method of amplification taught by Shi et al comprises a first selective amplification of a gene region of interest using forward and reverse primers, followed by a step of removing remaining outer primers using exonuclease I, and a subsequent amplification of a portion of the gene having a mutation using forward and reverse primers nested within the primers used for the first amplification. The reference further teaches analysis of the amplified products using SSCP by denaturing the amplification product to produce single stranded polynucleotides that are allowed to adopt particular conformations and the detection of mutations based on mobility of the strands through a substrate (p.273 – SSCP analysis; p.271, right col., last paragraph; Fig. 1).

The art of Liu et al teaches a method for the bidirectional PCR amplification of specific alleles (Bi-PASA) for the detection of mutations (Figure 1A; col.4 lns. 30-51). The reference teaches that amplification is carried out using region specific forward and reverse primers (identified as 'P' and 'Q' in the reference) which provide a positive control (col. 4 lns.53-54), as well as allele specific primers (identified as 'A' and 'B' in the reference) which are selective for the mutant and wildtype alleles of the analyzed gene (col.4 lns.54-57). The reference further teaches that the method can be performed using all four primers in one reaction, or as separate reactions using different combinations of useful primers (col.5 lns 31-36), and specifically teaches the analysis of reactions containing primers PQB or PQA (e.g.: Fig. 3A lanes 16 and 17, respectively). The reference teaches incorporation of a radioactive label into the amplified nucleic acid

for purposes of analysis of the amplification products (col.3 lns.24-30; Fig 2; col.6 ln.15), and analysis of amplification products by electrophoresis (col.6 lns.15-17).

It would be prima facie obvious to use the methods of Shi et al and Liu et al to perform the mutation analysis methods claimed by 10/695,089. Using the methods of Shi et al and Liu et al to analyze the PM1 and PM2 mutations of *Brassica* would result in the methods claimed in the instant application. Furthermore, such methods would include using the specific primers recited in claims 4, 5, 14, 15, and 17 (i.e. SEQ ID NO: 11, 12, 15, 16, and 18) and the broadly recited primers of claims 2, 3, 12, 13, 24, and 25 (i.e. primers 'having a sequence as set forth in' the recited positions of the recited SEQ ID NOs) as Shi et al and Liu et al teach using primers flanking the analyzed site, as well as allele specific primers with an allele specific nucleotide at the 3'-end.

This is a provisional obviousness-type double patenting rejection.

Response to Remarks

Applicants have acknowledged the rejection of claims for double patenting, and expressed that they are willing to file a terminal disclaimer upon indication of allowability.

The rejection is **MAINTAINED.**

Conclusion

12. None of the presently presented claims are allowable; no claims are free of the prior art.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephen Kapushoc whose telephone number is 571-272-3312. The examiner can normally be reached on Monday through Friday, from 8am until 5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached at 571-272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

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